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P	L	S	S	I	F	S	R	I	G	D	P
CCC	ATA	TCG	TCA	ATC	TTC	TCG	AGG	ATT	GGG	GAC	CCT
	T										

Fig. 1

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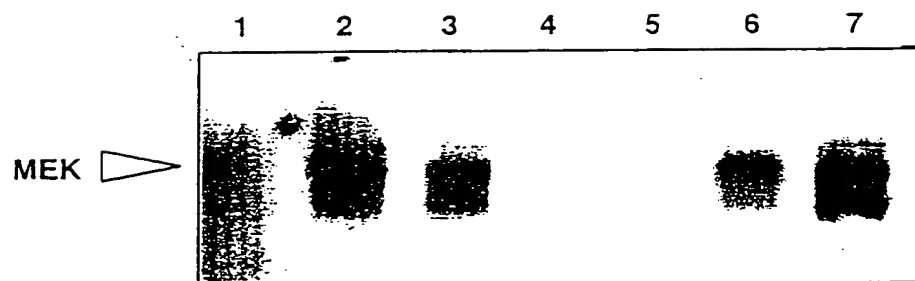


Fig. 2

Conservation of PreS2-TLM between various HBV subtypes

Nucleotide sequences, amino acid sequences and hydropathy values of the amino acid side chains (according to Kyte & Doolittle, 1982) of PreS2-TLM from subtype ayw (1) as compared to six other HBV subtypes ayw (2), adr (1), adr (2), ayr, adw and adw2. The amino acids identical with the sequence of PreS2-TLM of subtype ayw (1) and the associated hydropathy values are shown in boldface.

Subtype ayw (1)

CCC	TTA	TCG	TCA	ATC	TTC	TCG	AGG	ATT	GGG	GAC	CCT
Pro	Leu	Ser	Ser	Ile	Phe	Ser	Arg	Ile	Gly	Asp	Pro
-1.6	3.8	-0.8	-0.8	4.5	2.8	-0.8	-4.5	4.5	-0.4	-3.5	-1.6

Subtype ayw (2)

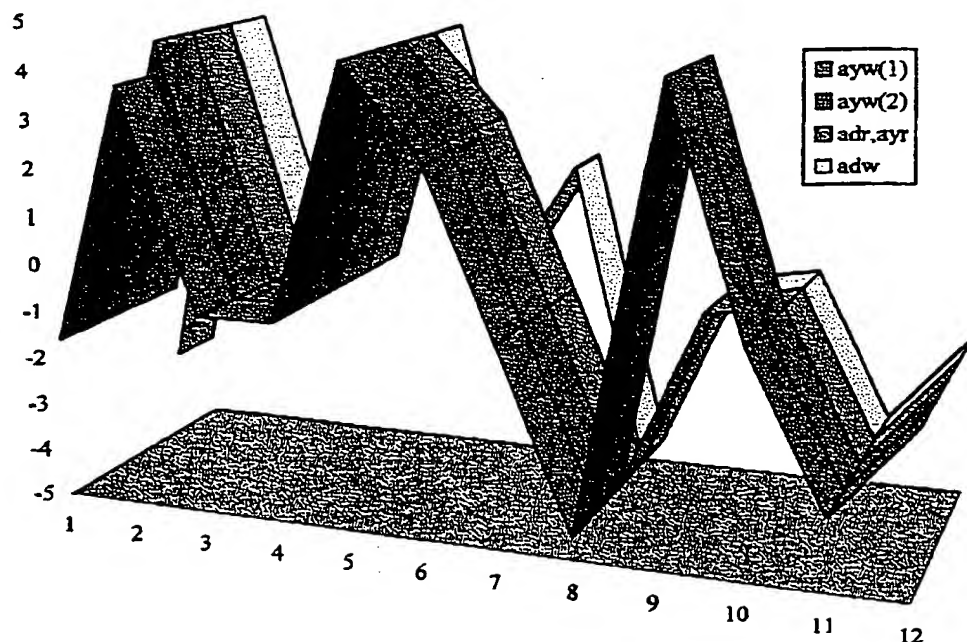
CCC	ATA	TCG	TCA	ATC	TTC	TCG	AGG	ATT	GGG	GAC	CCT
Pro	Ile	Ser	Ser	Ile	Phe	Ser	Arg	Ile	Gly	Asp	Pro
-1.6	4.5	-0.8	-0.8	4.5	2.8	-0.8	-4.5	4.5	-0.4	-3.5	-1.6

Subtypes adr (1), adr (2), ayr

CCC	ATA	TCG	TCA	ATC	TTC	TCG	AGG	ACT	GGG	GAC	CCT
Pro	Ile	Ser	Ser	Ile	Phe	Ser	Arg	Thr	Gly	Asp	Pro
-1.6	4.5	-0.8	-0.8	4.5	2.8	-0.8	-4.5	-0.7	-0.4	-3.5	-1.6

Subtypes adw, adw2

CAC	ATC	TCG	TCA	ATC	TCC	GCG	AGG	ACT	GGG	GAC	CCT
His	Ile	Ser	Ser	Ile	Ser	Ala	Arg	Thr	Gly	Asp	Pro
-3.2	4.5	-0.8	-0.8	4.5	-0.8	1.8	-4.5	-0.7	-0.4	-3.5	-1.6



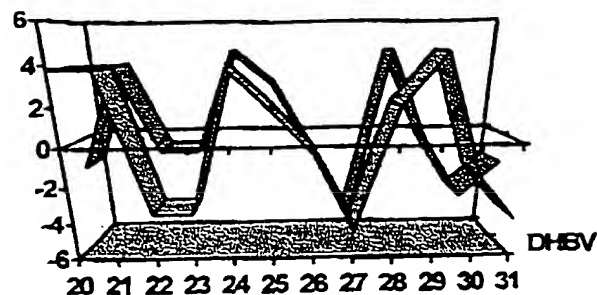
Amphiphilia of PreS2-TLM in various HBV subtypes

Representation of the distribution of the hydrophilic and hydrophobic amino acids in PreS2-TLM in subtype ayw (1) (blue) and the other HBV subtypes ayw (2) (green), adr (1), adr (2) ayr (red) as well as adw and adw2 (gray). The hydropathy values of the amino acid side chains (according to Kyte & Doolittle, 1982) are plotted on the y-axis, positive values correspond to hydrophobic amino acid side chains and negative values correspond to hydrophilic ones. The 12 amino acids of PreS2-TLM and the corresponding sequences of six other subtypes are plotted on the x-axis, the N-terminal proline being located at position 1.

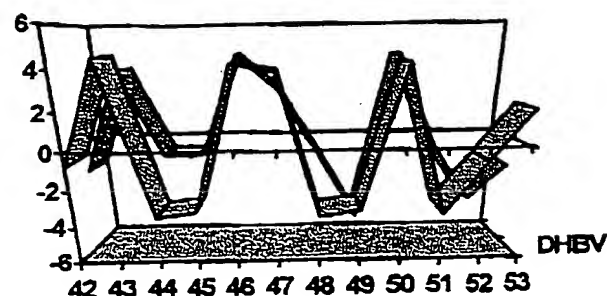
Fig. 3

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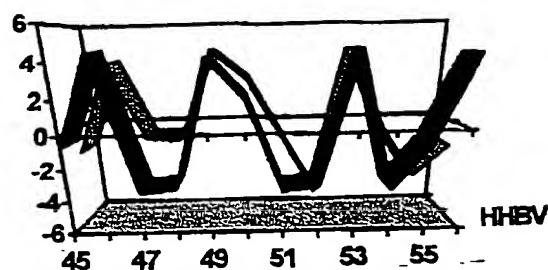
n	DHBV	PreS2-TLM	DHBV	PreS2-TLM
20	L (Leu)	P (Pro)	3,8	-1,6
21	L (Leu)	L (Leu)	3,8	3,8
22	N (Asn)	S (Ser)	-3,5	-0,8
23	Q (Gln)	S (Ser)	-3,5	-0,8
24	L (Leu)	I (Ile)	3,8	4,5
25	A (Ala)	F (Phe)	1,8	2,8
26	G (Gly)	S (Ser)	-0,4	-0,8
27	R (Arg)	R (Arg)	-4,5	-4,5
28	M (Met)	I (Ile)	1,9	4,5
29	I (Ile)	G (Gly)	4,5	-0,4
30	P (Pro)	D (Asp)	-1,6	-3,5
31	K (Lys)	P (Pro)	-3,9	-1,6



n	DHBV	PreS2-TLM	DHBV	PreS2-TLM
42	T (Thr)	P (Pro)	-0,7	-1,6
43	I (Ile)	L (Leu)	4,5	3,8
44	D (Asp)	S (Ser)	-3,5	-0,8
45	H (His)	S (Ser)	-3,2	-0,8
46	V (Val)	I (Ile)	4,2	4,5
47	L (Leu)	F (Phe)	3,8	2,8
48	D (Asp)	S (Ser)	-3,5	-0,8
49	H (His)	R (Arg)	-3,2	-4,5
50	V (Val)	I (Ile)	4,2	4,5
51	Q (Gln)	G (Gly)	-3,5	-0,4
52	T (Thr)	D (Asp)	-0,7	-3,5
53	M (Met)	P (Pro)	1,9	-1,6



n	HHBV	PreS2-TLM	HHBV	PreS2-TLM
45	T (Thr)	P (Pro)	-0,7	-1,6
46	I (Ile)	L (Leu)	4,5	3,8
47	Q (Gln)	S (Ser)	-3,5	-0,8
48	H (His)	S (Ser)	-3,2	-0,8
49	V (Val)	I (Ile)	4,2	4,5
50	M (Met)	F (Phe)	1,9	2,8
51	D (Asp)	S (Ser)	-3,5	-0,8
52	H (His)	R (Arg)	-3,2	-4,5
53	I (Ile)	I (Ile)	4,5	4,5
54	D (Asp)	G (Gly)	-3,5	-0,4
55	S (Ser)	D (Asp)	-0,8	-3,5
56	V (Val)	P (Pro)	4,2	-1,6



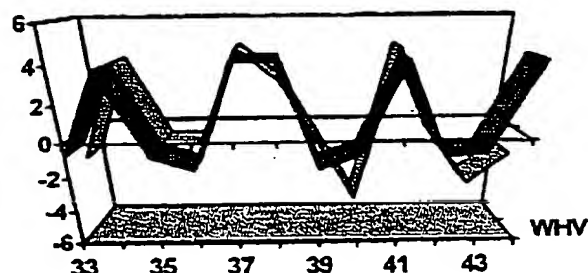
Amphiphilic motives in the PreS region of various avian hepadnaviruses

Comparison of the hydropathy profiles of PreS2-TLM (green) with segments of the PreS region of DHBV3 (red) and HHBV (blue). The positions of the amino acids in DHBV3 and HHBV are indicated in the tables (n) together with the amino acid sequence of the corresponding segment and the PreS2-TLM. The hydropathy values are also indicated (according to Kyte & Doolittle, 1982). Motives having a distribution of hydrophobic and hydrophilic amino acids similar to that in PreS2-TLM are found between amino acids 20 to 31 and 42 to 53 of DHBV3 or 45 to 56 of HHBV.

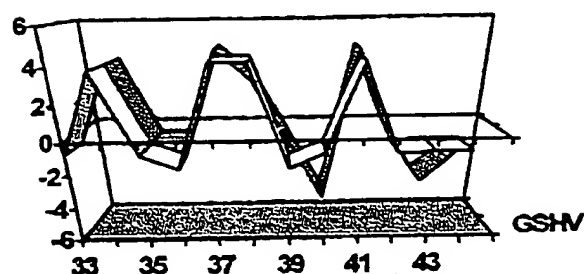
Fig. 4

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n	WHV	PreS2-TLM	WHV	PreS2-TLM
33	T (Thr)	P (Pro)	-0,7	-1,6
34	L (Leu)	L (Leu)	3,8	3,8
35	S (Ser)	S (Ser)	-0,8	-0,8
36	P (Pro)	S (Ser)	-1,6	-0,8
37	V (Val)	I (Ile)	4,2	4,5
38	V (Val)	F (Phe)	4,2	2,8
39	P (Pro)	S (Ser)	-1,6	-0,8
40	T (Thr)	R (Arg)	-0,7	-4,5
41	V (Val)	I (Ile)	4,2	4,5
42	S (Ser)	G (Gly)	-0,8	-0,4
43	T (Thr)	D (Asp)	-0,7	-3,5
44	I (Ile)	P (Pro)	4,2	-1,6



n	GSHV	PreS2-TLM	GSHV	PreS2-TLM
33	T (Thr)	P (Pro)	-0,7	-1,6
34	L (Leu)	L (Leu)	3,8	3,8
35	S (Ser)	S (Ser)	-0,8	-0,8
36	P (Pro)	S (Ser)	-1,6	-0,8
37	V (Val)	I (Ile)	4,2	4,5
38	V (Val)	F (Phe)	4,2	2,8
39	P (Pro)	S (Ser)	-1,6	-0,8
40	T (Thr)	R (Arg)	-0,7	-4,5
41	V (Val)	I (Ile)	4,2	4,5
42	S (Ser)	G (Gly)	-0,8	-0,4
43	T (Thr)	D (Asp)	-0,7	-3,5
44	T (Thr)	P (Pro)	-0,7	-1,6

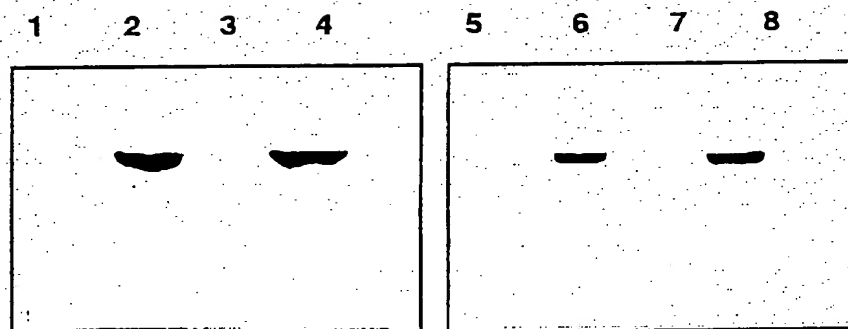


Amphiphilic motives in the PreS2 region of various hepadnaviruses of rodents

A comparison of the hydropathy profiles of PreS2-TLM (green) with segments of the PreS region of WHV (turquoise) and GSHV (yellow). The positions of the amino acids in WHV and GSHV are indicated in the tables (n) together with the amino acid sequence of the corresponding segment and the PreS2-TLM. The hydropathy values are also indicated (according to Kyte & Doolittle, 1982). Motives having a distribution of hydrophobic and hydrophilic amino acids similar to that in PreS2-TLM are found between amino acids 33 to 44 of PreS2 from WHV and GSHV. The motive is conserved between both hepadnaviruses.

Fig. 5

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DHBV42-53-EGFP is a cell-permeable protein

Immunoblot of cytosolic lysates of 293 cells after 10 minutes (1, 2, 5, 6) or 20 minutes of incubation (3, 4, 7, 8) with 1 mM EGFP (1, 3, 5, 7) or 1 mM DHBV 42-53-EGFP (2, 4, 6, 8). For carrying out the immunoblot, an antibody directed against the N-terminal hexa-His-Tag of the recombinant proteins (1-4) or directed against EGFP was used (5-8).

Fig. 6